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group consisting of stroke, angina and acute respiratory distress.

Please add the following claims.

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28. A method for treating acute respiratory distress in a mammal, comprising administering to the mammal an effective amount of SNO-Hb[FeII] or SNO-Hb[FeIII].

29. A method for causing vasodilation in a mammal, comprising administering to the mammal SNO-Hb(FeII).

REMARKS

Claims 1-3 and 6-8 have been canceled. Claims 9, 12, 15, 20 and 21 have been amended. Claims 28 and 29 have been added.

Support for Claim 28 is found on page 13, lines 33-35 of the specification, for example. Support for Claim 29 can be found on page 12, lines 22-25, page 12, lines 32-33, and page 13, lines 10-12.

At page 28, line 28, the word *precipitable* has been corrected to *soluble*, as it is evident to one of skill in the art that this is the intended word that provides consistency with the properties of low molecular weight molecules. In many enzymatic assays in molecular biology, a low molecular weight molecule becomes covalently bound to a high molecular weight molecule, or a high molecular weight molecule is cleaved to release a low molecular weight molecule. The high and low molecular weight products can be separated by acid precipitation (such as by trichloroacetic acid), whereby a precipitated high molecular weight product can be collected on a filter and the low molecular weight product can be collected as the filtrate. Alternatively, the low molecular weight product can be collected in the supernatant after centrifugation to pellet insoluble

(precipitated high molecular weight) material. See, for an example of an assay employing trichloracetic acid precipitation, Ausubel, F.M., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., containing supplements up through Supplement 40, January, 1998, pp. 18.7.4-18.7.5, especially "Support Protocol 2" on pages 18.7.14 and 18.7.15 (Exhibit 1).

Rejection of Claims 1-4, 6-21 and 23-27 Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 6-21 and 23-27 have been rejected under 35 U.S.C. § 112, first paragraph (Office Action on page 2), because they are said to be "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

More particularly (point A on page 2 of Office Action) the term "low" in Claims 1-4, 11, 14, 15 and 17 is said to be "a relative term which renders the claim indefinite."

Low molecular weight molecules and *small molecules* are terms of art in biology used to distinguish such molecules from *high molecular weight molecules* or *macromolecules*. See pages 6 and 7 of Lewin, B. *Genes V*, Oxford University Press, Oxford (1994), especially Table 1.1 and text explaining it; copy provided as Exhibit 2. In red blood cells, the most important naturally-occurring nitrosothiols are the low molecular weight molecule S-nitrosoglutathione and the macromolecule S-nitrosohemoglobin.

Low and high molecular weight molecules can be distinguished by the processes commonly used to purify proteins, as one of ordinary skill in the art knows. Dialysis tubing allows passage of low molecular weight molecules while retaining molecules of high molecular weight. Column chromatography based on separation by size allows the largest molecules to elute first; low molecular weight components are retarded in the commonly used size-separation chromatography media such as Sepharose.

A further method to distinguish those molecules called *high molecular weight* and *low molecular weight* is by their

precipitation properties. High molecular weight molecules are precipitable in trichloroacetic acid (commonly used at about 5% final concentration), while low molecular weight molecules remain soluble (see Exhibit 1).

See page 8, lines 21-32 of the specification for a more specialized discussion of "low molecular weight nitrosating agents." See also page 28, lines 26-28, wherein "low molecular weight S-nitrosothiols" are described as being trichloroacetic acid "precipitable." In the amendments to the specification, "precipitable" is being corrected to "soluble." It should be apparent to one of ordinary skill in the art that what was meant here was that the assay was for low molecular weight thiols (high molecular weight thiols do not cross the cell membrane) and that the assay for these low molecular weight thiols involved separation from high molecular weight components by trichloroacetic acid precipitation of the high molecular weight molecules and recovery and assay of the low molecular weight molecules in a supernatant. (See beginning of REMARKS section for further explanation.)

Claim 7 is said to be indefinite (points B, C and D on pages 2-3 of Office Action) in reciting several terms: *selected for rapid entry into the target cell, nitrosating agent, rapid,* and *the target cell.* Claim 7 has been canceled.

Claim 6 is said to be indefinite (point E on page 3 of Office Action). Claim 6 has been canceled.

Claim 8 is said to be indefinite (points F and G on page 3 of Office Action). Claim 8 has been canceled.

Claims 9 and 12 are said to be indefinite (point H on page 3 of Office Action) in that the recitation of "A preparation" is confusing. Claims 9 and 12 have been amended. Support for the amendments can be found at page 13, line 30, to page 14, line 3 and page 17, lines 12-17.

Claim 15 is said to be indefinite (point I on page 3 of Office Action) in the recitation of "selected for the oxidation

state of the heme iron and for the oxygen state" and in the recitation of "the oxygen state" without proper antecedent basis. The phrase "the oxygen state" refers to whether oxygen occupies the heme iron binding site (that is -- oxy or deoxy state) and "the oxidation state of the heme iron" refers to the oxidation state of the heme Fe atom itself (that is -- Fe^{2+} or Fe^{3+} (MetHb)). See the specification on page 10, line 29, to page 11, line 1. Also see page 12, lines 9-15.

Claim 15 is also said to be indefinite in that the phrase "selected for the oxidation state of the heme iron and for the oxygen state" in the claim "is indefinite as to the means and criteria for selection...." Claim 15 has been amended to more clearly define the invention.

Claims 15-27 are said to be indefinite (point J on page 3 of Office Action) "as to the mode of administration and the administrative amounts." Modes of administration and doses of SNO-hemoglobins are described on page 17, lines 3-9 of the specification. Specific examples of modes of administration and doses are found in Example 5 on pages 31-33, wherein experiments on rats are described. Doses producing similar effects in other animals and in humans can be extrapolated from the doses of cell-free hemoglobins tested in Example 5.

Compositions comprising SNO-Hb(FeII)O_2 were infused intravenously into dogs at doses described in Example 7. See page 35, lines 16-19 and page 35, lines 28-29. From these experiments, one of ordinary skill in the art would be able to extrapolate to doses effective in humans, and in other mammals.

Claims 20 and 21 are said to indefinite in the recitation of "a form of SNO-Hb" (point K on page 3 of Office Action). Claims 20 and 21 have been amended to eliminate this recitation.

Rejection of Claims 1-3, 6-15, 18-21, 26 and 27 Under 35 U.S.C. 102(b), or in the Alternative, Under 35 U.S.C. 103(a)

Claims 1-3, 6-15, 18-21 26 and 27 have been rejected (Item 5, page 4 of Office Action) under 35 U.S.C. 102(b) "as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Stamler *et al.*, WO 93/09806 (5/93)." Claims 1-3 and 6-8 have been canceled.

Stamler *et al.* (WO 93/09806) disclose S-nitroso-proteins, in particular, S-nitroso-tPA, S-nitroso-BSA, S-nitroso-cathepsin B, S-nitroso-lipoprotein and S-nitroso-immunoglobulin, and methods for producing the same. They also report a method which they claim results in the synthesis of S-nitroso-hemoglobin. However, this compound was not produced by any method reported in WO 93/09806, as attested to in the Declaration of Jonathan S. Stamler submitted with this Amendment. Methods used to synthesize other S-nitroso-proteins dissociated the hemoglobin subunits, oxidized the heme Fe and rendered the product useless for carrying oxygen. Therefore, WO 93/09806 does not teach how to make and use the invention.

The Examiner states (page 5, lines 3-10 of Office Action) that using equimolar amounts of nitrosating agent and hemoglobin, or using excess nitrosating agent would either anticipate or render obvious, respectively, Applicant's methods of synthesizing SNO-Hb[FeII]O_2 and SNO-Hb[FeII]. As explained in the Declaration of Jonathan S. Stamler, the hemoglobin compounds of Claims 10, 11, 13 and 14 were not made by any method described in, or implied by, WO 93/09806.

It was also reported in WO 93/09806 that S-nitroso-tPA and S-nitroso-BSA inhibit platelet aggregation and induce vasorelaxation. S-nitroso-cathepsin was demonstrated to induce vessel relaxation. S-nitroso-lipoprotein and S-nitroso-immunoglobulin showed platelet inhibition and vasodilatory effects. S-nitroso-BSA relaxed airway smooth muscle. Stamler *et al.* also propose that nitrosylated proteins be used to deliver NO to sites of the body (page 24, lines 10-12). The physiological

effects attributed to S-nitroso-hemoglobin in WO 93/09806 on page 19, line 22 to page 20, line 24, were not demonstrated therein. Whatever product, if any, may have been made by processes described in Example 19 (pages 58-59 of WO 93/09806) was not tested for any physiological properties. It cannot be assumed that the S-nitrosylation of hemoglobin will convert a protein which is, by itself, a vasoconstrictor, into a vasodilator. Figure 4A and Example 4A of the specification (see page 29, lines 5-13) show that SNO-Hb[FeII]O_2 is not a vasodilator.

Rejection of Claims 1-27 Under 35 U.S.C. § 103(a)

Claims 1-27 have been rejected (Item 6, page 6 of Office Action) under 35 U.S.C. § 103(a) as being "unpatentable over Stamler *et al.* in view of Feola *et al.*, U.S. Patent No. 5,439,882 ..., Klatz *et al.*, U.S. Patent No. 5,395,314 ... and Hunter, U.S. Patent No. 5,152,979 (10/92)." Claims 1-3 and 6-8 have been canceled.

The teachings of Stamler *et al.* (WO 93/09806) have been described above. The Declaration of Jonathan S. Stamler being filed concurrently with this Amendment states facts that lead to the conclusion that no SNO-oxyHb or SNO-deoxyHb could have been produced by the method described in Example 19 of WO 93/09806. The description provided in WO 93/09806 cannot be used to make and use SNO-oxyHb or SNO-deoxyHb.

Feola *et al.* (U.S. 5,439,882) describe cross-linked mammalian hemoglobin, a method of making the same, and a method of using the same as a blood substitute. Reduced glutathione is used in this reference to stop the cross-linking of hemoglobin when using o-adenosine as a cross-linking agent; in this case glutathione becomes part of the cross-linked hemoglobin compound. See column 13, lines 2-6 and lines 27-30. The reported function of glutathione is as an "oxidant trap" (column 13, lines 7-14). Feola *et al.* do not teach or suggest S-nitroso-hemoglobin.

Klatz *et al.* (U.S. 5,395,314) describe an apparatus and a method to preserve organs in a cadaver or in a brain-dead patient

before the organs can be removed for transplanting. The method employs a solution containing perfluorocarbons, which are to act as a blood substitute and transport oxygen in a manner similar to oxygen transport by hemoglobin. The solution may also contain antioxidants as free radical scavengers. Klatz *et al.* do not teach or suggest any modified form of hemoglobin.

Hunter (U.S. 5,152,979) describes a method for treating vascular obstructions, including those which may be caused by infection, sickle cell crisis, malaria and myocardial infarction. The method is to administer to a patient a surface active copolymer of a certain class of hydrophobes to reduce surface tension and friction in blood vessels, thereby reducing the incidence of thrombosis. Hunter does not teach or suggest the use of any type of any form of hemoglobin.

The Examiner states that the Stamler reference "teaches that nitrosating agents would be successful to achieve the desired effects of blood substitutes and also act as effective hemoglobin oxygen transporters." This conclusion cannot be drawn from WO 93/09806. According to statements in the Declaration of Jonathan S. Stamler, WO 93/09806 does not teach any modified form of hemoglobin that could act as an oxygen transporter. The attempted synthesis of S-nitrosohemoglobin failed. No reference cited in the rejection, other than WO 93/09806, mentions any form of S-nitrosohemoglobin. The specification describes the first successful synthesis of S-nitrosohemoglobin. See, for example, the specification at page 19, line 25 to page 20, line 16, and page 18, line 26 to page 19, line 6. Therefore, no combination of the cited references can render the invention obvious.

As for Claim 5, which does not require the direct use of synthesized S-nitrosohemoglobin, none of the cited references teaches or suggests the method of directly treating a patient's red blood cells with any compound. Hunter suggests the systemic administration of hydrophobic compounds, not S-nitrosothiols, to treat malaria.

CONCLUSION

In view of the above amendments and remarks, the Examiner is respectfully requested to reconsider and withdraw the rejections. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

Carol A. Egner

Carol A. Egner
Attorney for Applicant
Registration No. 38,866
Telephone (781) 861-6240
Facsimile (781) 861-9540

Lexington, Massachusetts 02173
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